

Requirements

for (di)sulfides analysis

on BDD electrodes

180.7074, Edition 4, 2022



Warning Symbol



The warning sign denotes a warning. It calls attention to a procedure or practice which, if not adhered to, could result in costs, damage or destruction of parts or all of the equipment. Do not proceed beyond a warning sign until the indicated conditions are fully understood and met.

For research purposes only. The ALEXYS system is not tested by the manufacturer to comply with the In Vitro Diagnostics Directive.

Observe safety

Operation of an electrochemical detector can involve the use of hazardous materials including corrosive fluids and flammable liquids. The instrument should only be operated by users with the following expertise:

- Completed degree as chemical laboratory technician or comparable vocational training
- Fundamental knowledge of liquid chromatography
- Knowledge and experience in the safe handling of toxic and corrosive chemicals and knowledge of the application safety measures prescribed for laboratories.
- Participation in an end-user training (daily use of system and chromatography software) performed by the manufacturer or a company authorized by the manufacturer.



Unskilled, improper, or careless use of the instrument and the related chemicals can create fire hazards, or other hazards which can cause death, serious injury to personnel, or severe damage to equipment and property.

Observe all relevant safety practices at all times.

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CHAPTER 1

Introduction

Thank you for ordering an ALEXYS LC-ECD system. For a successful on-site installation of the **disulfides analysis** on the ALEXYS system, please arrange the following requirements at your location in advance:

- a computer (see document 195.7000 for the PC requirements)
- general laboratory conditions and facilities, consumables and chemicals for use with the ALEXYS system (see document 180.7070C)
- application specific chemicals and consumables (see this document)



Arrange these requirements well in advance before the installation to prevent (costly) delays.

For LC-ECD applications, only chemicals of sufficient specific quality should be used to be able to have an optimal system with good performance. The appendix shows detailed descriptions of some of the chemicals that have been used in the Antec R&D laboratory, as an example of what works.



Have the chemicals and solutions ready at the start of the installation.

CHAPTER 2

Chemicals

Standards

Chemicals

- Standards of the components of interest in high purity grade
- Water (Resistivity >18M Ω ·cm, TOC<10ppb)
- Chemicals for preparation of real sample background (e.g. homogenization solution or microdialysis perfusion solution)

Preparation

Stock standard solutions

- 10 mL 1 mM of each individual standard in water
Store at 4 °C until use (max 1 day)

Solutions for peak identification

- 1 mL blank and 10 μ M of each single component
Store at 4 °C until use (max 1 day)

Calibrator solutions

- 0, 2, 4, 6, 8 and 10 μ M of the mixed standards
Store at 4 °C until use (max 1 day)

Mobile phase

Composition as used in application note 211-005	100 mM phosphoric acid 100 mM citric acid pH 3.0 500 mg/L octanesulfonic acid, sodium salt (OSA)
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Note 1: once loaded with ion-pairing agent (OSA), that column should be kept dedicated to this analysis. The reason is that the ion-pairing agent is difficult to wash off a column and will affect subsequent analyses when run without ion-pairing agent in the mobile phase.

Note 2: these conditions are suggested starting conditions and not guaranteed to give best results for all kinds of unknown samples. Condition optimisation may be necessary.

Chemicals

- Water (Resistivity >18M Ω ·cm, TOC<10ppb)
- Phosphoric acid (commercial solution of 85% w/v in water)
- Citric acid, monohydrate
- Octane sulphonic acid, sodium salt (OSA)
- 50% w/w NaOH in water (commercial solution)

Preparation

1. Add 0.8 litre water and a clean stir bar to a clean wide glass beaker and let it stir on a stirring plate. After addition of each component, let it stir until completely dissolved before adding the next component.
2. Add 6.86 mL 85% w/v phosphoric acid solution.
3. Add 21.0 g citric acid.
4. Set the pH of the mobile phase using the 50% NaOH solution.
5. Add 500 mg octane sulphonic acid, sodium salt
6. Add water to a total volume of 1 Litre.
7. Degas the mobile phase for 15 minutes in a sonic bath.

The mobile phase can be stored for 1 week at 4 °C and should be refreshed at least every 3 days or more often when it is in the system at room temperature.



Daily check the mobile phase bottle in the system for microbial growth. Refresh the mobile phase **at least once every 3 days** or more often if bacterial growth is observed earlier!



Do not filter the solutions by any means. The 0.2 μ m inline Whatman filters present in the low pressure solvent lines will take care of filtering.

System wash solutions

Chemicals

- Acetonitrile
- Water (Resistivity >18M Ω ·cm, TOC<10ppb)

Preparation

- 250 mL water, degassed (autosampler needle wash solution)
- 500 mL water, degassed (piston wash)
- 250 mL 20% acetonitrile, degassed (column flushing)
- 250 mL 50% acetonitrile, degassed (column flushing/storage)

A P P E N D I X

A list of general use chemicals with purity and purchase details is shown below as a guideline. The listed brands/purities are not necessarily the best chemicals, but these have been giving good results at the Antec R&D laboratory.

If for any reason alternative chemicals need to be purchased, be aware that chemicals that have a specification of high purity may have been tested for UV-active impurities, which can mean that they may still contain electrochemically active impurities. This is one of the reasons why 'HPLC grade' water is not recommended for use with EC detection:

- choose chemicals with the same purity or better
- do not choose ultra dry grade or anhydrous chemicals

Table 1. Brands and purities of chemicals used for application development at Antec.

Component	Purity	Brand	Order no:	Mw	Kg/L
Ortho-Phosphoric acid, 85% w/v in water	p.a.	Fluka	79620	98.00	D:1.68
Citric acid, monohydrate	p.a.	Acros	124910010	210.14	
1-Octane sulphonic acid, sodium salt (OSA)	HPLC grade	Acros	384771000	216.28	
Acetonitrile	HPLC grade, 99.9%	Acros	268260025	41.05	D:0.781
NaOH, 50% w/v in water	puriss., p.a., for HPLC; 50%	Fluka	71686	40.00	D:1.54
Water	TOC <10 ppb and deionised, resistivity >18 MOhm-cm (Barnstead Easypure II)				

Manufacturers/Vendors

Sigma-Aldrich	http://www.sigmaaldrich.com
Fluka	http://www.sigmaaldrich.com
Fisher Scientific	http://www.fishersci.com
Barnstead	http://www.thermoscientific.com